REMARKS

Claims

Claims 1–72 are pending of which claims 1–12, 15–17, 19–23, 26–41, 43, 64–66 and 68–72 are under examination pursuant to the restriction requirement mailed November 28, 2008 and Applicants' response thereto filed January 9, 2009 and clarification of May 20, 2009.

Claims 13, 14, 18, 24, 25, 42, 44–63 and 67 are withdrawn from consideration pursuant to the aforementioned restriction/election requirement.

Claims 73 and 74 are added by this paper.

Incorporation by reference under 37 CFR §1.57

Sequence listing

A revised sequence listing disclosing the protein sequence appearing as GenBank accession No. NP_004549 (UniProt accession No. Q99748) and the polynucleotide sequence which encodes it (GenBank Accession No. U78110) is enclosed herewith. With respect to the additional sequences incorporated into the present application, SEQ ID NO: 7 discloses the amino acid sequence of *Homo sapiens* neurturin preproprotein, as taught by Kotzbauer et al. (*Nature*, 384: 467-470, 1996). See paragraph [0013] of the published US specification (US pub. No. 2008-0241106). The cDNA sequence of *Homo sapiens* neurturin as taught by Kotzbauer (GenBank Accession No. U78110) appears as SEQ ID NO: 6 in the sequence listing. Although the GenBank accession of the polynucleotide sequence is not explicitly recited in the instant specification, such was taught by the Kotzbauer et al. and was moreover cross-referenced with the aforementioned GenBank accession No. NP_004549. Printouts of Kotzbauer et al. and GenBank accession No. NP_004549 are enclosed herewith for the Examiner's review.

The amino acid sequence for human neurturin and the cleaved product thereof was published before the earliest priority date of the instant application (Kotzbauer et al. published on December 5, 1996; GenBank accession date: May 7, 1997; UniProt accession date: May 1, 1997). The NP_004549 sequence and the disclosure in the Kotzbauer article (including the polynucleotide molecules recited therein) were incorporated by reference into the present specification. See paragraph [0104] of the published US specification. It is respectfully submitted that the material being inserted is the material previously incorporated by reference and that the amendment contains no new matter. Favorable action is earnestly solicited.

Applicants further note that the actual protein sequence relating to NP_004549 has not undergone any revisions since the first accession thereof in the database (Rev. 1 vs. Rev. 93 in

the UniProt database). The same is true for the accessioned sequence in the NCBI database. See, for example, a sample of the sequence comparison in the enclosed Exhibit A. For a detailed analysis, the Examiner is referred to the following database entries:

(1) http://www.uniprot.org/uniprot/Q99748?version=*

(2) http://www.ncbi.nlm.nih.gov/sviewer/girevhist.cgi?val=NP_004549+

Although not necessary because the instant claims are directed to method(s) of using neurturin polypeptides of the instant invention, Applicants furthermore aver that the neurturin polynucleotide sequence of SEQ ID NO: 6 is identical to the polynucleotide sequence accessioned in GenBank as U78110, which is explicitly taught by the aforementioned Kotzbauer et al. as human neurturin cDNA sequence. See the legend of Fig. 3 of Kotzbauer et al. The entirety of disclosure in the Kotzbauer reference was incorporated by reference into the present application. Accordingly, the amendment to the specification incorporating the human neurturin nucleic acid sequence does not raise new matter.

Claim amendments

Purely to facilitate prosecution, claims 1 and 6 have been limited to the use of neurturin products of the instant application.

Claim 4 has been amended in accordance with standard US lexical practice.

Claim 10 has been amended as per the Examiner's suggestion.

Claims 17 and 18 have been amended to recite sequence identifier numbers of the human neurturin protein and cDNA sequences. Support for the amendment can be found in, for example, paragraphs [0013] and [0029] of the published specification (US pub. No. 2008-0241106). See also the disclosure in Kotzbauer et al. (*Nature* 384: 467-470, 1996) and the reference thereto at paragraph [0013] of the published specification. See *supra* with regard to incorporation by reference under 37 CFR §1.57. See also *Capon v. Eshhar*, 418 F.3d 1349 (Fed.Cir. 2005).

The amendment to claim 17 with regard to recitation of percent sequence identity to a given polypeptide sequence (i.e., SEQ ID NO: 7) is supported by the disclosure in, for example, paragraph [0031] of the published specification. Claim 18, which was previously withdrawn, is now directed to the elected invention, e.g., methods of using a neurturin polypeptide product. Inclusion thereof in the examined claim set is respectfully requested.

New claims 73 and 74 are identical to claim 17, except that the subject matter of these claims is directed to the use of a narrower genus of polypeptide molecules. Support for the

new claims can be found in, for example, paragraph [0031] of the published specification.

It is respectfully submitted that the amendments presented herein do not raise new matter. Entry thereof is respectfully requested.

Restriction/Election

Page 4 of the Restriction Requirement mailed November 28, 2008 outlined a detailed election of species requirement consisting of more than six Markush Groups. Insofar as the PTO fails to provide any rationale as to why search/examination of anything *beyond* a single species would constitute an undue burden for each of the Markush groups, it is submitted that the nebulous requirement is without merit. Purely in order to facilitate prosecution, the following species/nested species were elected with traverse:

(a) Applicants elected embryonic stem cells as recited in present claims 3 and 36;

(b) Applicants elected regeneration of insulin-producing cells. See, claim 6;

(c) Applicants elected diabetes type I as recited in present claims 11, 12 and 33;

(d) Applicants elected pancreatic diseases, as recited in present claim 27;

(e) Applicants elected insulin production in response to glucose. See, claim 39;

(f) Applicants elected parenteral administration. See, claim 67.

Applicants respectfully request reconsideration of this exhaustive election of species requirement. At least, there should be minimal search burden to examine all the species under (a) [i.e., 2 species], (c) [i.e., 3 species], (e) [i.e., 2 species] and (f) [i.e., 3 species], as recited in the aforementioned claims 3, 11, 39 and 67 (a maximum total of 36 *possible* outcomes). The administrative guidelines under MPEP §803 expressly states that "If search and examination of an entire application can be made without serious burden, the examiner *must* examine it on the merits, even though it includes claims to independent or distinct invention (Emphasis added)." The same is respectfully requested.

It is further submitted that the specification teaches that each of the aforementioned species are generic to the claimed embodiment of the instant invention. To this end, with respect to the species of cells in (a), the specification discloses that the types of cells recited in present claim 3 are pluripotent cells whose meaning is well-appreciated in the art (e.g., capable of differentiating into a diverse range of specialized cells). As such, the requirement that Applicants elect a single type of cells is without legal and scientific merit. The same is true for the diseases recited in present claim 11. As explicitly stated in the paragraphs bridging pages 2 and 3 of the present specification, the three types of diabetic diseases (i.e.,

diabetes type I, latent autoimmune diabetes in adults (LADA), or progressed diabetes type II) are all characterized by a deficiency in functional beta cells. Additionally, Applicants submit the methods and/or routes of administration recited in the present claims are well-appreciated in the art. As such, the requirement that Applicants elect a single type of route of administration is respectfully traversed.

Expansion of search

Applicants additionally submit that should the election of species requirement still be maintained, in accordance with proper Markush practice, should no prior art be found which renders the invention of the elected species unpatentable, the search of the remainder of the generic claim(s) should be continued in this same application. To this end, Applicants note that in accordance with the decisions in *In re Weber*, 580F.2d 455, 198 USPQ 328 (CCPA 1978) and *In re Haas*, 580 F.2d 461, 198 USPQ 334 (CCPA1978), it would improper for the Office to refuse to examine that which applicants regard as their invention, unless the subject matter in a claim lacks unity of invention. See MPEP §803.02 in accord.

Applicants reserve the right to file one or more divisional applications directed to non-elected inventions.

IDS

Copies of non-patent literature documents are enclosed herewith for the Examiner's review.

Specification

The specification has been amended to incorporate sequence identifier numbers (SEQ ID NO) of the polypeptide/polynucleotide molecules disclosed therein. The specification has been further amended to cross-reference the revised sequence listing included herein. It is respectfully submitted that the amendments to the specification do not recite new matter. Entry thereof is respectfully requested.

Applicants are in the process of preparing a substitute specification incorporating all the aforementioned changes. It is respectfully requested that the objection to the specification be held in abeyance until such can be furnished. See MPEP §714.

Formal matters

Claim 10 has been amended as per the Examiner's suggestion, rendering the objection

thereof moot. Withdrawal of the objection is respectfully requested.

Rejection under §112, ¶2

The rejection of claim 17 under this section is rendered moot by the foregoing amendments. More specifically, claim 17 in its amended form recites uses of proteins having 70% sequence homology to SEQ ID NO: 2 or the cleaved product thereof. Thus the metes and bounds of the proteins and/or variants thereof is clear to those skilled in the art. Withdrawal of the rejection is respectfully requested.

The Examiner is thanked for the careful reading of claims 26–29. The foregoing amendments render the rejection thereof moot. Withdrawal of the rejection is respectfully requested.

Rejections under 35 USC §112, ¶1

Claims 1-12, 15-17, 19-23, 26-41, 43, 64-66 and 68-72 are rejected under 35 USC §112, ¶1, as allegedly failing to comply with the written description/enablement requirement(s). This rejection is respectfully traversed.

At the outset, it should be noted that Applicants have amended claims 17 and 18 to recite structural details of the biomolecules of the present invention. Thus under PTO's own Written Description guidelines, the claims are in compliance with the statutory requirements under §112, ¶1 with respect to written description. See the discussion provided *infra*. The following comments are provided to rebut the rejection of the broader claims under §112, ¶1.

Written description

At the outset Applicants submit that the claims have been amended to recite neurturin products. This is not to imply that the scope of the original claims was problematic under US law. For example, modulators (e.g., agonists and antagonists) and effectors of neurturin are well-described by the disclosure in the original specification. Accordingly, Applicants' amendment of the claims is not to be construed with acquiescence to this or any other ground of rejection.

As to the written description of "neurturin products" presently recited in the claims, the original specification expressly teaches that such include purified natural or synthetic neurturin and variants thereof comprising insertion, substitution and deletion or chemical modifications therein and recombinant proteins, for example, hybrids of neurturin and other TGF-beta proteins (preferably from the GDNF-family). The specification explicitly teaches that

neurturin products are substantially homologous to the human neurturin precursor protein having the amino acid sequence published as GenBank Accession Number NP_004549, including, for example, mouse and human neurturin sequences taught by Kotzbauer et al. Further encompassed by the term are neurturin homodimers or heterodimers of a neurturin protein product and another protein, wherein the other protein preferably belongs to the GDNF-family. As shall be clear from the discussion, the specification provides more than adequate written description for the genus of neurturin products claimed herein.

Neurturin products containing neurturin

At least two neurturin family members were appreciated in the art prior to the earliest priority date of the instant application, e.g., mouse and human neurturin sequences, as taught by the aforementioned Kotzbauer et al. The respective polynucleotide sequences of mouse and human neurturin were also known and publically accessioned in GenBank by Kotzbauer et al. See *supra*.

Neurturin products containing neurturin variants

Variants are described in the specification as containing insertion, substitution, deletion or chemical modification in the core neurturin sequence. A skilled worker who is provided with the cDNA sequences encoding human or mouse neurturin polypeptide and is further knowledgeable of PCR methodology for creation of one or more variations in the core sequence can routinely make and test such variants without undue experimentation. Representative examples are provided in the Examples section of the instant specification. Regarding various neurturin protein variants, Applicants submit that neurturin exerts its effects via activation of specific receptor complexes, especially a complex consisting of two GFR-α-2 and two c-Ret tyrosine kinase molecules. Highly related GDNF protein family members of neurturin, GDNF and artemin bind to similar but distinct receptor complexes (reviewed, for example, in Airaksinen and Saarma (2002); Zariola and Saarma (2003)). The regions important for receptor complex binding in GDNF family members have been mapped and were found to be separated by loops which are less important for receptor binding (Baloa et al.; Parkash et al. 2008). It can be deduced from these experimental results or references, respectively, that changes (e.g. amino acid exchanges) can be made in neurturin without substantial loss of receptor affinity and thereby without loss of biological activity (Baloa et al., 2000). Thus variant molecules compising one or more substitutions, insertions, deletions or chemical modifications could be routinely made and used in accordance with the present invention.

Homologs of neurturin (70%, 90% or 95% homology to human neurturin sequence)

Applicant has reviewed the PTO's new *Written Description Guidelines* and amended the claims in accordance with Example 11B beginning on Page 39 of the *Training Materials* (Rev. 1, March 25, 2008). While applicants may not agree with the agency's interpretation of the elements necessary to meet the statutory requirements of 35 U.S.C. § 112, ¶1, nonetheless, the pending claims have been amended to substantially conform to these.

The PTO's example provides a claim to a polynucleotide having the nucleic acid sequence of SEQ ID NO: 1, which encodes the polypeptide of SEQ ID NO: 2. The polypeptide of SEQ ID NO: 2 has a novel activity Y. This conforms to Applicants' present invention wherein the claimed neurturin polynucleotide of SEQ ID NO: 6 (GenBank Accession No. U78110) encodes a protein of SEQ ID NO: 7 which has a well-characterized activity (for example, ability to stimulate and/or induce the differentiation of insulin producing cells from progenitor cells). The exemplary claims are as follows:

Claim 1. An isolated nucleic acid that encodes a polypeptide with at least 85% amino acid sequence identity to SEQ ID NO: 2.

Claim 2. An isolated nucleic acid that encodes a polypeptide with at least 85% amino acid sequence identity to SEQ ID NO: 2; wherein the polypeptide has activity Y.

The guidelines state that both claims 1 and 2 satisfy the requirements set forth under §112, ¶1. With respect to claim 1, it is stated that "Although the recitation of a polypeptide with at least 85% identity represents a partial structure...the disclosure of SEQ ID NO: 2 combined with the knowledge in the art regarding the genetic code would put one in possession of the genus of nucleic acids that encode SEQ ID NO: 2. Further, with the aid of a computer, one could list all of the nucleic acid sequences that encode a polypeptide with at least 85% sequence identity with SEQ ID NO: 2. Additionally, the level of skill and knowledge in the art is such that one of ordinary skill would be able to use conventional sequencing and nucleic acid synthesis techniques to routinely generate and identify nucleic acids that encode the polypeptide of SEQ ID NO: 2, as well as those that encode any polypeptide having 85% structural identity to SEQ ID NO: 2. Thus, one of ordinary skill in the art conclude that the applicant would have been in possession of the claimed genus at the time of filing."

With respect to claim 2, it is stated that "[although] the specification fails to teach which of the nucleic acid sequences that encode a polypeptide with at least 85% sequence identity to SEQ ID NO: 2 encode a polypeptide having the required activity Y,... the

specification identifies domains responsible for activity Y." Such a disclosure is evident from the disclosure contained in Applicants' specification. For example, the present specification discloses at least two types of neurturin proteins, mouse and human neurturin, which are about 91% homologous to one another. See, paragraph [0031] of the published specification. Information on the primary structure (i.e., polypeptide sequence) and amino acid residues that are important in biological activity of neurturin was appreciated by the skilled worker prior to the filing date of the instant application. See, for example, Fig. 3B of the cited article by Kotzbauer et al. (*Science*, 1996).

The guidelines further states that "Although all conservative amino acid substitutions in these domains will not necessarily result in a protein having activity Y, those of ordinary skill in the art would expect that many of these conservative substitutions would result in a protein having the required activity. Thus, a correlation exists between the function of the claimed protein and the structure of the disclosed binding and catalytic domains [such that] based on the applicant's disclosure and the knowledge within the art, those of ordinary skill in the art would conclude that the applicant would have been in possession of the claimed genus of nucleic acids based on the disclosure of the single species of SEQ ID NO: 1."

Thus, it is evident that the specification clearly provides the information set forth by the U.S. Patent Office as needed to meet the statutory requirements under §112, ¶1. Withdrawal of the rejection is respectfully requested.

Hybrids, homodimers and heterodimers of neurturin with itself or other proteins

With respect to neurturin products containing GDNF, paragraph [0012] of the published specification explicitly teaches that GDNF proteins were structurally and functionally characterized by Lin et al. (*Science*, 260:1130-2, 1993). A copy of Lin is enclosed herewith for the Examiner's review. Methods of making hybrids (e.g., chimeric proteins) containing neurturin and GDNF (either in homodimeric form or heterodimeric form) was appreciated by any molecular biologist. Similar methods could be employed to conjugate neurturin to other TGF-β family of proteins, which were well-appreciated in the art as of the filing date of the instant specification. Representative examples are provided in paragraph [0013] of the published specification. The skilled worker may refer to the cross-referenced publications by Takahashi (*Cytokine Growth Factor Rev*, 12(4):361-73, 2001), USP 6,090,778 and EP1005358B1, the disclosures in which were incorporated in their entirety into the instant specification.

The PTO's contention that the disclosure of specific examples of use of neurturin

polypeptides fails to provide adequate written description for the genus of the neurturin products is misplaced. Firstly, this is not a case like that in *University of California v. Lilly*, 964 F.2d 1128 (Fed.Cir. 1997) or *University of Rochester v. Searle*, 358 F.3d 1303 (Fed.Cir. 2004) where functional language was involved with insufficient structural details available for a chemical compound. Rather, here, the proteins of the present invention well-described with *both* their structural features (e.g,, human and mouse neurturin and/or hybrids thereof with TGF-β family members such as GDNF) and functional features (e.g., ability to stimulate and/or induce the differentiation of insulin producing cells from progenitor cells). Thus, these facts are similar to those in *Capon v. Eshhar*, 418 F.3d 1349 (Fed.Cir. 2005) and *Falkner v. Inglis*, 448 F.3d 1357 (Fed.Cir. 2006). In these cases, the court held that even where there are no examples within the scope of a claimed genus, a written description exists where the elements of the members of the genus are known. Here, the human and mouse neurturin proteins and hybrids thereof with TGF-β family are well-characterized, and the level of skill and knowledge in the art of molecular biology at the time of filing was such that production of chimeras and hybrids with such biomolecules was conventional. Inasmuch as the amino acid sequences are clearly described, and the meaning of the term variants, hybrids, heterodimers, and homodimers is appreciated in the art, the written description of antibody molecules having the claimed binding properties is clear. This shows "possession" (written description) of multiple members of the genus and thus provides a written description for the claimed uses thereof, in compliance with the requirements set forth under §112, ¶1.

Furthermore, a skilled worker reviewing the disclosure in the specification and the art references cited therein is provided with significant guidance to finding other members of the genus of neurturin products and testing for the claimed activity without undue experimentation. For example, the ability of variant proteins to stimulate or induce the differentiation of insulin producing cells from progenitor cells could be carried out using the assays described in Example 4 of the original specification. This is more than sufficient to provide the necessary possession of the invention. In this regard, the examiner is also referred to the enclosed documents. As can be seen, the literature recognizes the kind of experimental result provided in the current application, and, hence, possession of the claimed genus.

It is therefore courteously submitted that Appellants' claims 1-12, 15-17, 19-23, 26-41, 43, 64-66 and 68-72 in the current form fully comply with the statutory requirements of 35 U.S.C. §112, ¶1 with respect to written description. Withdrawal of the rejection is respectfully requested.

Rejection under 35 U.S.C. §112, ¶1 (enablement)

Regarding the lack of enablement rejection of claim 1, Applicants courteously submit that the specification, coupled with a skilled worker's knowledge, provides adequate guidance to make and use the neurturin products of the instant invention without undue experimentation. See *Genentech Inc. v. Novo Nordisk* A/S, 108 F.3d 1361 (Fed. Cir. 1997) (quoting *In re Wright*, 999 F.2d 1557 (Fed. Cir. 1993)). For example, the present application provides polypeptide sequences (SEQ ID NO: 7, homologs thereof and hybrids, homodimers and heterodimers thereof with TGF-β family members such as GDNF). Techniques for obtaining variant neurturin polypeptides and hybrids thereof were well-described in literature. See, for example, the disclosure contained in the Examples and the references cited therein. Such may include, for example, recombinant techniques (as exemplified) as well as chemical synthetic means that are known in the art. To this end, variant polypeptides which are encoded by polynucleotide sequences that share, for example, 70% homology with SEQ ID NO: 7 could be routinely generated via synthesis and design of DNA constructs and/or vectors that encode such polypeptides. Such techniques were known in the art. In accordance with Applicants' own disclosure, the skilled worker could utilize known techniques (for example, conserved substitutions) to generate polynucleotides that share at least 90% or 95% homology with SEQ ID NO: 6, transform a host cell (for example, eukaryotic cell) with the variant polynucleotide sequence, thus generating a repertoire of polypeptide molecules. These variant polypeptides could then be routinely tested with regard to their ability to stimulate progenitor cells into insulin producing cells, for example, using the assays described in Applicants' own specification. For example, the disclosure in Example 4 provides for methods of transforming cells with constructs encoding neurturin. The disclosure in Example 5 outlines methods for the functional characterization of the transformed cells. As outlined in Example 6, sophisticated *in vivo* assays may be further employed. Thus a genus of molecules which satisfy the claimed structural and/or functional elements can be isolated and tested. The whole process would constitute, at most, routine experimentation.

In light of this detailed disclosure, the courts have placed the burden on the PTO to show otherwise. It is courteously submitted that the Examiner has not presented any evidence to refute the findings or the conclusions made herein. In addition, no evidence has been presented to support the contention that the claimed neurturin products could not be made and used, in a manner that is commensurate with Applicants' claimed invention.

With respect to the variant neurturin proteins, Applicants invite the Examiner to review a recent precedential opinion issued by the United States Board of Patent Appeals and Interferences (*Ex parte* Kubin, Appeal No. 2007-0819, BPAI 2007), a copy of which is enclosed herewith.

The facts in Kubin are applicable to the present case. In Kubin, the Examiner contended that "at least 80% identity language" in the absence of any working examples, other than a few representative species, fails to provide enablement of the claimed genus of molecules. See, page 10 of *Ex parte* Kubin. The Examiner alleged that specification did not teach "which 20% . . . of amino acid residues should be changed in order to maintain the biological functions." In response, Appellants argued that the specification disclosed "in detail how to: 1) make variants of SEQ ID NOs: 1 and 2; 2) calculate the percent identity between SEQ ID NOs: 1 and 2 and the variant sequence; and 3) test the variant sequence to determine [functional activity]." See, items 23 and 24 at page 13. Appellants further argued that in view of the high level of skill in molecular biology, methods of making the claimed nucleic acid sequences and screening for activity [were] known in the art and described in the specification and that the "experimentation involved to produce other sequences within the scope of the claims" and thus to practice the full scope of the claims would have been "well within the skill of those in the art." The amount of experimentation involved would have been routine and not undue. See, items 27–30 at page 14.

Likewise in the present application, Applicants disclose a genus of neurturin products, which are, for example, neurturin proteins, variants thereof, and hybrids thereof with other TGF-β family members such as GDNF. Methods of obtaining other polypeptide sequences, for example, sequences that are 70% homologous to SEQ ID NO: 7, were all routine. The same is true for the chimeric proteins containing GDNF and neurturin. Therefore, the level of "experimentation involved to produce other [molecules] within the scope of the claims" and thus to practice the full scope of the claims would have been "well within the skill of those in the art." Favorable action is earnestly solicited.

Methods of using the claimed neurturin products

The Examiner cites Johnson et al (US patent No. 5,739,309; filed: October 8, 1997) and Wobus et al. (US patent pub. No. 2005-0054102; filed: November 21, 2006) to assert that the claimed methods for using the neurturin products are non-enabled. At the outset, Applicants respectfully submit that the references relied upon by the Examiner are especially inept in the face of the showing that the state of the art pertaining to polypeptide molecules of the

present invention, protein formulations, and use of such molecules in therapeutics are all mature. It should be noted in this context that Johnson is fully seven years before the earliest priority date of the instant application (i.e., November 27, 2003) and fails to take into account the rapid progress made in the post-genomic era. Wobus examines the ability of Pdx1, Pax4, Pax6, ngn3, Nkx6.1, Nkx6.2, Nkx2.2, HB9, BETA2, NeuroD, Isl1, HNF1-alpha, HNF1-beta, HNF3, or a combination thereof to differentiate stem cells into insulin-producing cells. Contrary to the PTO's assertions, Wobus does not refute Applicants' assertion of enablement, but rather supports it. See *infra* for a detailed discussion thereof.

With regard to the methods claimed herein, Applicants submit that the experiments described in the present application involving the differentiation of Pax4 over-expressing mouse embryonic stem cells represent a general model system for beta-cell differentiation from non-beta cells. The specification teaches that the differentiation of stem cell or progenitor cell into a specialized cell type such as pancreatic beta cells recapitulates, at least in part, the differentiation program occurring during embryonic development. See, for example, Edlund et al. (2002), Dor and Melton (2008), Guo and Hebrok (2009), Champeris-Tsaniras and Jones (2010). Representative examples in this context are presented by the articles by Xu et al. (2008) and Dor (2008), which demonstrate the presence of a population of progenitor cells in the adult mouse pancreas which have the ability to generate new endocrine cells (including insulin producing beta cells). The progenitor cells are characterized by the expression of transcription factor ngn3, in their dormant state, and activate a large number of known embryonic beta cell differentiation regulatory genes during differentiation (Xu et al. 2008).

Differentiation of beta cells from human ductal tissue via expression of embryonic pancreatic differentiation genes such as Pdx1 has also been demonstrated previously (e.g. Bonner-Weir 2000). Recapitulation of embryonic differentiation pathways has also been found to be a key issue in successful differentiation of embryonic and other stem cells (D'Amour et al. 2006; Spence and Wells 2007; Kroon et al. 2008; Champeris Tsaniras 2010; Prince and Kinkel 2010).

The Pax4 over-expression induced in the model system presented herein serves as a facilitator of beta cell differentiation. Pax4 is a key transcription factor which controls the differentiation of beta cells during mammalian embryogenesis (Sosa-Pineda et al. 1997; Edlund 2002; Oliver-Krasinski and Stoffers 2008). Separately, Pax4 expression can also facilitate differentiation of mouse ES cells (Lin et al. 2007), mouse and human ductal pancreatic progenitors (Noguchi et al., 2006). Pax4 may also confer differentiation of human embryonic

stem cells towards a beta cell phenotype (Lieu 2008). Transfection with certain transcription factors is commonly used in the field as an initial method to induce certain states or to accelerate differentiation in stem cells or differentiating cells, e.g. transdifferentiation or pluripotency (Takahashi and Yamanaka 2008). Subsequently, the expression of such factors can be induced via expression-inducing small molecules or growth factors, or in some cases over-expression can be replaced with a recombinantly expressed cell-transducing transcription factor protein (Noguchi et al., 2003; Lu et al. 2007; Chen et al. 2006/2007; Shi et al. 2008; Zhou et al. 2009; Champeris Tsaniras and Jones 2010). Such techniques are well-characterized in the art. Importantly, small molecules and growth factors which can induce the expression of the Pax4 gene in rodent and human cells have been described (Mussmann et al 2007; Brun et al., 2008). These publications indicate that the stable Pax4 over-expression can be replaced by altered culture conditions or addition of Pax4-inducing substances.

Therefore the results presented by the present application are not only limited to the specific mouse embryonic stem cell line with ectopic Pax4 over-expression described in the present application but can also be applied to the differentiation of other stem or progenitor cell types including not genetically modified cells of human origin.

Finally, as regards Johnson (USP 5,739,307) and Wobus (US 2005-0054102), the following comments are provided:

Johnson et al. describe a pro-differentiation function of neurturin for neuronal cells and certain other cell types for which gene expression data suggest a possible role for this protein, including bone marrow. Pancreatic beta cells are not described in Johnson et al. Developmentally, pancreatic beta cells are not neuronal cells, and are not derived from or related to other tissues, cells or organs described in Johnson et al. as expressing neurturin and therefore likely to be affected by neurturin. Instead, pancreatic beta cells are derived from endoderm during development, making them fundamentally different from the cell types described in Johnson et al., especially neurons. See, for example, Slack et al. (1995), Edlund et al. (2002), Oliver-Krasinski and Stoffers et al. (2008). It is thus concluded that the subject matter of the present application differs from that of Johnson's disclosure. The same is true of Wobus et al. To this end, the Examiner's attention is drawn to Example 4 of the instant application, which shows the induction of differentiation of insulin-producing cells by neurturin. The data presented by the present application demonstrate the neurturin can significantly promote and enhance ES cell differentiation into insulin-producing cells compared to wild-type ES cells. Accordingly, it is submitted that Applicants' disclosure provides more

than sufficient guidance to objectively enable one of ordinary skill in the art to make and use the claimed invention with an effort that is routine within the art.

Withdrawal of the rejection under 35 U.S.C. §112, ¶1, is respectfully requested.

The Commissioner is hereby authorized to charge any fees associated with this response to Deposit Account No. 13-3402.

Respectfully submitted,

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